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INVESTIGATION OF THE CHEMICAL NATURE OF HUMIC ACIDS  
(HYDROLYSIS OF HUMIC ACIDS AND EXPRESS METHODS FOR  
DETERMINING THE FUNCTIONAL GROUPS)

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ABSTRACT

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Humic acids derived from peat and two soil types have been subjected to acid hydrolysis to determine the structural modifications induced by acid hydrolysis; the functional groups have been determined in both hydrolyzed and nonhydrolyzed humic acids by several techniques, including a rapid method developed by the authors, which in comparison with longer conventional methods discloses fair reliability. Principally, the average carbon-nitrogen ratio and bond between the humic acid molecules and nitrogenous compounds are affected by hydrolysis. *Author*

INTRODUCTION

Academician V. R. Vil'yams (ref. 2) has indicated that ulmic and humic 151\* acids undergo an irreversible conversion under the influence of protracted boiling with water. Considerable interest attaches to the study of the irreversible changes of humic acids during boiling with mineral acids. In this case, in addition to hydrolysis of the nitrogenous compounds and carbohydrates loosely bound with the humic acid molecule, a more profound modification of the humic acids themselves can be anticipated. In view of the paucity in the literature of data on the change in humic acids during acid hydrolysis and the almost complete lack of data pertaining to the modification of the radicals in hydrolysis, we felt it necessary to attack this problem. The investigation of the hydrolysis of humic acids is of practical as well as theoretical significance.

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\*Numbers in the margin indicate pagination in the original foreign text.

In an investigation of the soils of the USSR, N. P. Remezov (ref. 8) has shown that to each soil type there corresponds a characteristic average C : N ratio. From this point of view, it is important in studying the humic acids to know this ratio, on the one hand, and, on the other, to know the nature and strength of the bond between the humic acids and nitrogenous compounds. It has been demonstrated in the work of S. S. Dragunov and E. F. Bakhtina that when various peat humic acids are heated with 5 and 25% hydrochloric acid, the quantity of hydrolyzed nitrogenous substances and the content of nonhydrolyzed nitrogen in the humic acids is essentially independent of the acid concentration.

This implies that a part of the nitrogenous substances are loosely bound with the humic acid molecule, while a certain part of the nitrogen enters into the humic acid molecule and cannot be detached without decomposition of the latter. In this respect, soil humic acids behave similarly to the peat variety (ref. 4).

In studying the composition of soil humus according to the procedure of I. V. Tyurin (ref. 10), humic acids are derived from 0.1-n NaOH after cold processing of 0.1-n  $H_2SO_4$ , then after heating for five hours with 1.0-n  $H_2SO_4$ . The question arises as to the difference between these humic acid fractions.

In the investigation of peats according to the method of the Peat Institute, peat specimens are heated with 2% HCl after the extraction of bitumens in order to hydrolyze the hemicelluloses, and humic acids are extracted from the residue with alkali. In our investigations of peats, we subscribed to an alternate method, to wit: After removal of the bitumens and water-soluble substances prior to hydrolysis, we extracted the humic acids, which were then subjected to investigation, since we have always maintained that acid hydrolysis would detach both the nitrogenous substances and the carbohydrates that are loosely

compounded with the humic acids and, hence, would modify them.

Furthermore, we have not overlooked the possibility of a certain qualitative and quantitative change in the functional radicals, as well as the kernel of the humic acids themselves if acid hydrolysis is performed in the presence of a large number of extraneous, more or less easily hydrolyzable substances: hemi-celluloses, polyuronides, etc. /152

Desiring to investigate the possible structural modifications occurring in acid hydrolysis, we determined the elementary composition and investigated by various techniques the content of functional groups for nonhydrolyzed and hydrolyzed humic acids derived from the same soil and peat specimens.

#### EXPERIMENTAL PART

The humic acids were extracted from chernozem (black earth), podzolic turf, and peat by a method described in a paper by the authors (ref. 4). The chernozem specimen was obtained from the Kursk region (Central State Chernozem National Forest). The specimen of podzolic turf was obtained from fir-tree soil from the Upper Moscow River National Forest, a horizon, depth 4-15 cm. The peat specimen, a complex upper horizon variety with 30% decomposition, came from the Galitskiy Mokh bogs.

Hydrolysis was carried out according to the following procedure.

A weighed portion of the humic acid was boiled in a round-bottomed flask for 24 hours (100 ml 5% HCl per gram of humic acid). After cooling, the humic acid was filtered off, washed in water, and dessicated, the filtrate and wash water were collected together, the total nitrogen content was determined by the Kjeldahl method, the monoamino acids and nitrogen of the bases were determined. For this purpose, a 10% solution of phosphotungstic acid was added dropwise to 100 ml of the hydrolyzate, which was then left standing until the next day,

when it was filtered and the residues washed. In the residue, as well as in the filtrate and wash water combined, the Kjeldahl nitrogen content was determined.

The results obtained are summarized in tables 1 and 2.

Table 1 shows the total quantity of hydrolyzed substances and the carbohydrate content in the hydrolyzate.

TABLE 1  
RESULTS OF HYDROLYSIS OF HUMIC ACID  
IN PERCENTAGE OF ORGANIC SUBSTANCE IN SAMPLE

Humic Acid	Total Soluble Substances in Hydrolyzate	Carbohydrates
From peat	19.9	9.50
From chernozem	30.2	10.25
From podzol	40.6	10.04

The quantity of carbohydrates passing into the hydrolyzate is almost the same for all humic acids, but the total quantity of substances passing into the hydrolyzate is considerably larger for the soil humic acids, which is explained by the higher content of nitrogenous compounds. Moreover, under the conditions of hydrolysis, the humic acids themselves tend somewhat to be dissolved.

For all of the humic acids, the percentage of monoamino acids in the hydrolyzate was 64-75 (table 2). This indicates, on the one hand, the albuminous origin of these nitrogenous compounds and, on the other, their highly facile hydrolyzability to monoamino acids, whence we postulate that these substances were either very easily hydrolyzed products of the decomposition of albumins or that they were primarily amino acids bound with the humic substances.

TABLE 2

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## DESCRIPTION OF NITROGENOUS COMPOUNDS

Humic Acid	Nitrogen content in % of organic substance in humic acid		In hydrolyzate, regarding nitrogen in hydrolyzate as 100%	
	in initial sample	after hydrolysis	monoamino acids	Substances precipitated by phospho- tungstic acid
From peat	1.72	0.97	74.9	10.6
From chernozem	4.04	2.65	64.0	35.6
From podzol	4.86	1.79	75.0	25.0

Comparing the data (table 3) for non hydrolyzed and hydrolyzed humic acids from peat and chernozem, we note an increase in the carbohydrate content for the hydrolyzed humic acids, a certain decrease in the hydrogen content, and a substantial increase in the nitrogen content, hence a concomitant increase in the C : N ratio.

TABLE 3

## DATA FROM ELEMENTARY PERCENTAGE ANALYSIS OF HUMIC ACIDS

Humic acids	C	H	N	O	C/N
From peat; nonhydrolyzed.....	61.78	4.28	1.72	32.22	35.7
From peat; hydrolyzed.....	62.55	4.05	0.97	32.47	64.9
From chernozem; nonhydrolyzed..	57.32	4.25	4.04	34.39	14.3
From chernozem; hydrolyzed...	59.07	3.69	2.65	34.59	22.4

If we make the assumption that the nitrogen content in the hydrolyzed peat humic acid corresponds to one nitrogen atom, we can calculate an empirical formula and the molecular weight of the indicated humic acids (table 4).

TABLE 4

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## EMPIRICAL FORMULAS AND MOLECULAR WEIGHT OF HUMIC ACIDS

Humic acid	Empirical formula	Molecular weight
From peat; nonhydrolyzed....	$C_{88}H_{71}O_{33}N_2$	1659
From peat; hydrolyzed....	$C_{74}H_{68}O_{29}N$	1424
From chernozem; nonhydrolyzed..	$C_{89}H_{89}O_{30}N_4$	1387
From chernozem; hydrolyzed....	$C_{78}H_{68}O_{34}N_2$	1580

It is interesting to note that the molecular weight of humic acids turned out, according to the indicated calculation, to be near 1400, i.e., the value normally assumed for humic acids. For peat humic acid, the molecular weight after hydrolysis, as might be expected, was lowered, and for chernozem humic acid, on the other hand, an increase in the molecular weight is observed. A possible explanation for this effect could be inhomogeneous composition of the humic acids, as well as possible condensation processes accompanying the hydrolysis reaction.

We subjected the investigated humic acids to exhaustive methylation by treatment with methyl alcohol saturated with hydrogen chloride, dimethyl sulfate in a basic medium, and diazomethane in an ether solution. Both nonhydrolyzed and hydrolyzed humic acids were subjected to methylation. For a truer comparison of the methylation results between the hydrolyzed and nonhydrolyzed humic acids, we converted the data for the latter so that the percentage content of the functional groups would be calculated relative to the weighed portion after subtraction of the carbohydrates and albuminous substances removed in hydrolysis (percent N x 6.25), rather than the initial weighed portion (table 5).

TABLE 5

FUNCTIONAL GROUPS IN HUMIC ACIDS;  
PERCENTAGE OCH<sub>3</sub> CONTENT IN ORGANIC MATERIAL

	Initial methoxyl		Methanol + HCl			Dimethyl sulfate		Diazomethane		
			nonhydrol.			nonhydrol.		nonhydrol.		
			unconverted	converted	hydrolyzed	unconverted	converted	unconverted	converted	hydrolyzed
Humic acid										
From peat	1.86	1.75	9.84	11.64	9.25	10.06	11.83	18.02	21.39	21.07
From chernozem	1.17	0.73	9.01	11.47	8.06	3.28	4.10	7.52	9.47	18.18
From podzol	1.54	1.37	8.46	12.53	9.42	4.91	6.54	10.02	14.84	16.71



If we bear in mind that the carbohydrates and nitrogenous substances removed in hydrolysis cannot be methylated during treatment with methanol, we can say, after the indicated conversion, that the content of functional acid groups (carboxyl and acid hydroxyl) decreased by 2.4-3.4% for all of the investigated hydrolyzed humic acids. For the hydroxyl groups determined by treatment with dimethyl sulfate, on the other hand, an increase was observed for the hydrolyzed acids; for peat it was rather appreciable - 2.4%; for the other two humic acids the increase was much less, only 0.86-0.97%.

In the hydrolysis of humic acids, hydroxyl as well as carboxyl groups are liberated, so that the combined analysis of these groups by treatment with diazomethane would give higher results for the hydrolyzed humic acids. A 1.87% increase is observed for podzol humic acid, whereas for chernozem humic acid 155 the increase is 8.71%. In this case, we have not overlooked the possibility of new hydroxyl groups being produced in the hydrolysis process. Consequently, it must be supposed that in acid hydrolysis the humic acids undergo a more or less profound chemical modification and, in so doing, differ depending on the nature of the humic acid.

The consistency of the data from the diazomethane treatment of peat humic acid is attributable to the fact that during hydrolysis the content of carboxyl groups was lowered, while the content of hydroxyl groups increased in the same ratio. As demonstrated by our investigation, as well as those of Tishchenko and Rydalevskaya (ref. 9), Aleksandrova (ref. 1), Natkina (ref. 7), and others, the assessment of the functional groups in humic acids is significant in a great many respects.

However, the exhaustive methylation of humic acids is an entire research project unto itself, requiring several months to carry out.

One of the present authors (refs. 3 and 5) has proposed a method for rapidly determining the functional groups in humic acids. It was instructive to compare the results of methylation with the data obtained from this express method of analysis.

The procedure that we have developed enables one chemist with the properly modified apparatus to determine, in one working day, the content of carboxyl and hydroxyl groups for several humic acid specimens.

We determined the total content of the functional groups by titration. This method was developed by A. F. Dragunova and differs from the method of Ubaldini, as well as that of T. A. Kukharensko (ref. 6), in that boiling of the humic acid in a basic solution, which we view as undesirable, is avoided.

Analysis procedure. - A 0.02-0.04 g weighed portion of finely ground humic acid was placed in a 50 ml flask, the air was drawn out of the flask by nitrogen streaming,<sup>1</sup> 5 ml of 0.1-n NaOH was poured in, the flask was sealed with a stopper, and the humic acid was allowed to dissolve, after which dehydrated alcohol was added to the 50 ml mark, the mixture was left to stand for 15 min, and 20-25 ml were filtered off. Sodium nitrate coagulated in the alcohol solution and sedimented rather quickly.

10 ml of solution were used for the analysis and titrated with 0.01-n HCl. A similar blank test was run without the humic acid and the total content of functional groups in the humic acid was determined from the difference in titrations. The results were expressed in milliequivalents per gram of humic acid. The entire determination required about one hour.

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<sup>1</sup>The analysis had to be performed in an inert gas atmosphere when the humic acid was separated under the same conditions.

We analyzed the carboxyl groups by the amonia method.

Analysis procedure. - 0.1 g of finely ground humic acid was placed in a Würtz flask and the air drawn off by nitrogen streaming.<sup>1</sup> 10 ml of concentrated  $\text{NH}_4\text{OH}$  solution were added and time was allowed for the portion to dissolve, after which a vacuum was created in the flask and the surplus ammonia and water were driven off by heating the flask in a water bath at 50-70° until all moisture had been removed.

The flask was evacuated, nitrogen was passed through, and a small quantity of water was poured in; the ammonium humate dissolved rapidly in the water. To the ammonium humate solution were added 100 ml of 0.1-n HCl. The humic acid coagulated, and the clear solution was filtered off through an ordinary filter and washed with distilled water. The filtrate and wash water were placed in a flask for amonia distillation according to the method of Kjeldahl. The result-156 ant quantity of amonia corresponded to the content of carboxyl groups.

The results were expressed in milliequivalents per gram of humic acid.

The results of methylation are also expressed in milliequivalents per gram of dry and ash-free humic acid and are shown in table 6 for comparison.

As apparent from the data presented, the caboxyl groups determined by methylation and the ammonia method for peat and podzol humic acids give fair agreement; for chernozem, higher results are obtained by the ammonia method; for humic acid from chernozem, the data were high also for the sum of the carboxyl and hydroxyl groups, hence, based on the difference, similar results were obtained by comparable methods for the hydroxyl groups: 3.69 and 3.90 milliequivalents.

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<sup>1</sup>The analysis had to be performed in an inert gas atmosphere when the humic acid was separated under the same conditions.

TABLE 6

FUNCTIONAL GROUPS IN HYDROLYZED HUMIC ACIDS, AS DETERMINED BY  
ALTERNATE METHODS IN MILLIEQUIVALENTS PER GRAM OF ORGANIC SUBSTANCE

Humic acid	Carboxyl Groups		Carboxyl and hydroxyl groups		Hydroxyl groups based on difference	
	Methanol	ammonia method	diazomethane	titration method	3-1	4-2
From peat	3,11	3,47	7,45	8,27	4,34	4,80
From chernozem	2,68	5,37	6,37	9,27	3,69	3,90
From podzol	3,17	3,30	5,79	—	2,62	—

For the majority of humic acids extracted from different peats, determination of the carboxyl groups by the ammonia method and treatment with methanol result in good agreement, as shown by the work of S. S. Dragunov and S. F. Bakhtina (ref. 3). The humic acid was extracted in this case by 0.1-n NaOH in air, and the ammonia nitrogen was determined by the following procedure: The humic acid was treated with a 25% surplus aqueous solution of ammonia in air; the solution was evaporated until dry in a water bath, and the resultant ammonium humate was dried in a thermostat at 80°C. For analysis, a weighed portion of the ammonium humate was placed in a Claisen flask, water and magnesium oxide were added; the flask was connected to a vaporizer and the ammonia distilled off under reduced pressure (15-20 mm Hg) into a receptacle with titrated 0.1-n acid. The resultant data are summarized in table 7.

Consequently, the disparity in data between the methylation and ammonia 157 methods for humic acid from chernozem indicates that the analyzed groups has a special character in this humic acid.

TABLE 7

CARBOXYL GROUPS IN PEAT HUMIC ACIDS  
IN MILLIEQUIVALENTS PER GRAM OF ORGANIC SUBSTANCE

Peat humic acid	Methanol	Ammonia method	Difference
From hypnum bog .....	2,87	2,87	0,0
From sedge bog .....	2,70	2,61	0,09
From reed bog .....	2,82	2,61	0,21

Recognizing the good agreement between the methods compared in the case of peat humic acids as well as humic acid from forest podzol soils, it may be assumed that the accelerated method for determining the functional groups will find application in the study of soil humic acids, if only for obtaining a quick comparative characteristic.

#### CONCLUSIONS

1. In acid hydrolysis, humic acids undergo modification, principally with respect to the detachment of carbohydrates and weakly bound nitrogen compounds.
2. The content of the various functional groups in humic acids changes more or less appreciably following hydrolysis. The total content of the functional groups analyzed by diazomethane treatment increases for soil humic acids and remains unchanged for peat humic acid.
3. In the description and characteristics of humic acids it is important to note whether they were subjected to acid hydrolysis in the extraction process.
4. The C/N ratio, which is typical of various types of soils, changes abruptly for humic acids, depending on whether they have or have not been subjected to acid hydrolysis.

5. A procedure has been described for the express assessment of the functional groups in peat and soil humic acids.

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